

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for amplifying a microRNA molecule to produce DNA molecules, the method comprising the steps of:
  - (a) producing a first DNA molecule that is complementary to a target microRNA molecule using primer extension; and
  - (b) amplifying the first DNA molecule to produce amplified DNA molecules using a universal forward primer and a reverse primer.
2. The method of Claim 1, wherein at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule.
3. A method of Claim 1 wherein the primer extension uses an extension primer having a length in the range of from 10 to 100 nucleotides.
4. A method of Claim 1 wherein the primer extension uses an extension primer having a length in the range of from 20 to 35 nucleotides.
5. A method of Claim 1 wherein the extension primer comprises a first portion that hybridizes to a portion of the microRNA molecule.
6. A method of Claim 5 wherein the first portion has a length in the range of from 3 to 25 nucleotides.
7. A method of Claim 5 wherein the extension primer comprises a second portion.
8. A method of Claim 7 wherein the second portion has a length of from 18 to 25 nucleotides.
9. A method of Claim 7 wherein the second portion has a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO:1.
10. A method of Claim 1 wherein the universal forward primer has a length in the range of from 16 nucleotides to 100 nucleotides.

11. A method of Claim 1 wherein the universal forward primer consists of the nucleic acid sequence set forth in SEQ ID NO:13.

12. A method of Claim 7 wherein the universal forward primer hybridizes to the complement of the second portion of the extension primer.

13. A method of Claim 2 wherein the universal forward primer comprises at least one locked nucleic acid molecule.

14. A method of Claim 13 wherein the universal forward primer comprises from 1 to 25 locked nucleic acid molecules.

15. A method of Claim 1 wherein the reverse primer has a length in the range of from 10 nucleotides to 100 nucleotides.

16. A method of Claim 2 wherein the reverse primer comprises at least one locked nucleic acid molecule.

17. A method of Claim 16 wherein the reverse primer comprises from 1 to 25 locked nucleic acid molecules.

18. A method of Claim 1 wherein the reverse primer is selected to specifically hybridize to a DNA molecule complementary to a selected microRNA molecule under defined hybridization conditions.

19. A method of Claim 1 further comprising the step of measuring the amount of amplified DNA molecules.

20. A method of Claim 1 wherein amplification is achieved by multiple successive PCR reactions.

21. A method for measuring the amount of a target microRNA in a sample from a living organism, the method comprising the step of measuring the amount of a target microRNA molecule in a multiplicity of different cell types within a living organism, wherein the amount of the target microRNA molecule is measured by a method comprising the steps of:

- (1) producing a first DNA molecule complementary to the target microRNA molecule in the sample using primer extension;
- (2) amplifying the first DNA molecule to produce amplified DNA molecules using a universal forward and a reverse primer; and
- (3) measuring the amount of the amplified DNA molecules.

22. The method of Claim 21, wherein at least one of the universal forward primer and the reverse primer comprises at least one locked nucleic acid molecule.

23. The method of Claim 21, wherein the amount of the amplified DNA molecules are measured using fluorescence-based quantitative PCR.

24. The method of Claim 21, wherein the amount of the amplified DNA molecules are measured using SYBR green dye.

25. A kit for detecting at least one mammalian target microRNA comprising at least one primer set specific for the detection of a target microRNA, the primer set comprising:

- (1) an extension primer for producing a cDNA molecule complementary to a target microRNA, the extension primer comprising a first portion that hybridizes to a target microRNA and a second portion having a hybridization sequence for a universal forward PCR primer;
- (2) a universal forward PCR primer for amplifying the cDNA molecule, comprising a sequence selected to hybridize to the hybridization sequence on the extension primer; and
- (3) a reverse PCR primer for amplifying the cDNA molecule, comprising a sequence selected to hybridize to a portion of the cDNA molecule.

26. The kit according to Claim 25, wherein at least one of the universal forward and reverse PCR primers includes at least one locked nucleic acid molecule.

27. The kit according to Claim 25, wherein the extension primer has a length in the range of from 10 to 100 nucleotides.

28. The kit according to Claim 25, wherein the first portion of the extension primer has a length in the range of from 3 to 25 nucleotides.

29. The kit according to Claim 25, wherein the second portion of the extension primer has a length in the range of from 18 to 25 nucleotides.

30. The kit according to Claim 25, wherein the second portion of the extension primer has a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 1.

31. The kit according to Claim 25, wherein the universal forward PCR primer has a length in the range of from 16 to 100 nucleotides.

32. The kit according to Claim 25, wherein the universal forward primer consists of the nucleic acid sequence set forth in SEQ ID NO: 13.

33. The kit according to Claim 25, wherein the reverse PCR primer has a length in the range of from 10 to 100 nucleotides.

34. The kit according to Claim 25, wherein the reverse PCR primer comprises from 1 to 25 locked nucleic acid molecules.

35. The kit according to Claim 25, wherein the at least one mammalian target microRNA is a human microRNA.

36. The kit according to Claim 35, wherein the at least one target microRNA is selected from the group consisting of miR-1, miR-7, miR-9\*, miR-10a, miR-10b, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-18, miR-19a, miR-19b, miR-20, miR-21, miR-22, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-28, miR-29a, miR-29b, miR-29c, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-5p, miR-30e-3p, miR-31, miR-32, miR-33, miR-34a, miR-34b, miR-34c, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a, miR-99b, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-107, miR-122, miR-122a, miR-124, miR-124, miR-124a, miR-125a, miR-125b, miR-126, miR-126\*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-143, miR-144, miR-145, miR-146, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154\*, miR-154, miR-155, miR-181a, miR-181b, miR-181c, miR-182\*, miR-182, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189,

miR-190, miR-191, miR-192, miR-193, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a\*, miR-199a, miR-199b, miR-200a, miR-200b, miR-200c, miR-202, miR-203, miR-204, miR-205, miR-206, miR-208, miR-210, miR-211, miR-212, miR-213, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-220, miR-221, miR-222, miR-223, miR-224, miR-296, miR-299, miR-301, miR-302a\*, miR-302a, miR-302b\*, miR-302b, miR-302d, miR-302c\*, miR-302c, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-330, miR-331, miR-337, miR-338, miR-339, miR-340, miR-342, miR-345, miR-346, miR-363, miR-367, miR-368, miR-370, miR-371, miR-372, miR-373\*, miR-373, miR-374, miR-375, miR-376b, miR-378, miR-379, miR-380-5p, miR-380-3p, miR-381, miR-382, miR-383, miR-410, miR-412, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-429, miR-431, miR-448, miR-449, miR-450, miR-451, let7a, let7b, let7c, let7d, let7e, let7f, let7g, let7i, miR-376a, and miR-377.

37. The kit according to Claim 35, wherein the at least one target microRNA is selected from the group consisting of: miR-1, miR-7, miR-10b, miR-26a, miR-26b, miR-29a, miR-30e-3p, miR-95, miR-107, miR-141, miR-143, miR-154\*, miR-154, miR-155, miR-181a, miR-181b, miR-181c, miR-190, miR-193, miR-194, miR-195, miR-202, miR-206, miR-208, miR-212, miR-221, miR-222, miR-224, miR-296, miR-299, miR-302c\*, miR-302c, miR-320, miR-339, miR-363, miR-376b, miR-379, miR-410, miR-412, miR-424, miR-429, miR-431, miR-449, miR-451, let7a, let7b, let7c, let7d, let7e, let7f, let7g, and let7i.

38. The kit according to Claim 25, wherein the at least one target microRNA is a murine microRNA.

39. A kit for detecting at least one mammalian microRNA comprising at least one oligonucleotide primer selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO:499.

40. The kit according to Claim 39 comprising at least one or more oligonucleotide primers selected from the group consisting of SEQ ID NOS: 47, 48, 49, 50, 55, 56, 81, 82, 83, 84, 91, 92, 103, 104, 123, 124, 145, 146, 193, 194, 197, 198, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 239, 240, 247, 248, 253, 254, 255, 256, 257,

258, 277, 278, 285, 286, 287, 288, 293, 294, 301, 302, 309, 310, 311, 312, 315, 316, 317, 318, 319, 320, 333, 334, 335, 336, 337, 338, 359, 360, 369, 370, 389, 390, 393, 394, 405, 406, 407, 408, 415, 416, 419, 420, 421, 422, 425, 426, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 461 and 462.

41. An oligonucleotide primer for detecting a human microRNA selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 499.

42. An oligonucleotide primer according to Claim 41, wherein the primer is selected from the group consisting of SEQ ID NO: 47, 48, 49, 50, 55, 56, 81, 82, 83, 84, 91, 92, 103, 104, 123, 124, 145, 146, 193, 194, 197, 198, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 239, 240, 247, 248, 253, 254, 255, 256, 257, 258, 277, 278, 285, 286, 287, 288, 293, 294, 301, 302, 309, 310, 311, 312, 315, 316, 317, 318, 319, 320, 333, 334, 335, 336, 337, 338, 359, 360, 369, 370, 389, 390, 393, 394, 405, 406, 407, 408, 415, 416, 419, 420, 421, 422, 425, 426, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 461 and 462.